A08878

TOXICOLOGY AND APPLIED PHARMACOLOGY 14, 13-22 (1969)

The Metabolism of 1,1-Di(*p*-chlorophenyl)-2,2,2-trichloroethane and 1,1-Di(*p*-chlorophenyl)-2,2-dichloroethane in the Pigeon

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Received April 15, 1968

The Metabolism of 1,1-Di(*p*-chlorophenyl)-2,2,2-trichloroethane and 1,1-Di(*p*-chlorophenyl)-2,2-dichloroethane in the Pigeon. BAILEY, S., BUNYAN, P. J., RENNISON, B. D., and TAYLOR, A. (1969). *Toxicol. Appl. Pharmacol.* 14, 13-22. Pigeons have been fed diets containing 1000 ppm of the halogenated hydrocarbons, 1,1-di(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT) and 1,1-di(*p*-chlorophenyl)-2,2-dichloroethane (DDD) for 24 days. None of the birds fed either chemical died during the course of the experiment. Residues of these compounds and their metabolites were measured at intervals after the cessation of feeding and the results were treated statistically to determine rates of elimination or change from seven tissues. DDT was shown to be eliminated from all tissues at the same rate (half-life 28 days) and gave rise to DDE and DDD by separate pathways. DDD was shown to be eliminated from all tissues at a similar rate (half-life 24 days) and gave rise mainly to DDMU together with a very small amount of DDE. Large increases in average liver weights were noted in birds fed DDT. With the DDD-fed birds the increase in liver weight was very slight.

The insecticide 1,1-di(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT) and related compounds are widely used in agriculture and horticulture. This has resulted in the accumulation of residues in soils, in vegetation, and in wildlife. The determination of residues of DDT and its major metabolites in avian species is readily carried out, but the interpretation of the results obtained is difficult due to the lack of fundamental knowledge of the effect of DDT on birds and the lack of data on levels needed to cause death. A complicating factor is the large residue of organochlorine pesticides which accumulates in fat compared to levels in other tissues. It can be recirculated when these reserves are mobilized under starvation or other stress conditions.

There is a vast amount of information on the fate of DDT in numerous insect species starting with the work of Ferguson and Kearns (1949). It was later found (Perry and Hoskins, 1950; Sternburg and Kearns, 1950) that certain strains of house fly resistant to DDT had the ability to detoxify a large proportion of the applied DDT to 1,1-di(*p*-chlorophenyl)-2,2-dichloroethylene (DDE). Findings of metabolites of 1,1-di(*p*-chlorophenyl)-2-dichloroethane (DDD) in insects have also recently been reported (Gatterdam *et al.*, 1964).

The fate of DDT in vertebrates has also been investigated, but to a lesser extent. A recent review by Hayes (1965) summarizes the general state of knowledge regarding the

[1] Responsible for statistical analysis.

biotransformation of DDT. An extensive list of possible metabolic compounds that could be obtained from feeding DDT or its metabolites to rats has been given (Peterson and Robison, 1964), but not all the postulated compounds were isolated and identified. Evidence has also been produced (Pinto *et al.*, 1965) for the presence of *p,p'*-dichlorobenzhydrol and *p,p'*-dichlorodiphenylmethane as well as the more usual metabolites in rats. DDD was first proposed as a metabolite of DDT by Finley and Pillmore (1963), and it has been suggested (Barker and Morrison, 1964) that DDD appears as the result of bacterial postmortem decomposition. DDD has also been suggested (Jefferies and Walker, 1966) as a postmortem decomposition product in birds. Di(*p*-chlorophenyl)acetic acid (DDA) has been shown to be the final product in several mammals, but no such work has been reported on in birds.

During recent years many data have been collected on levels of pesticides occurring in wildlife, particularly birds, although the interpretation of such data is limited. Laboratory studies on the fate of DDT in birds are also limited. Stickel *et al.* (1966) found both DDE and DDD in cowbirds (*Molothrus ater*) fed DDT in the diet, but it was concluded that residue levels in most tissues were unsuitable for diagnostic purposes although levels of DDT and DDD in brain could be significant. The number of deaths that occurred in a very short time from fairly low levels of DDT feeding to cowbirds is noteworthy especially when compared with the low death rate reported later in our communication using pigeons. The absorption, metabolism and elimination of DDT in the common grackle (*Quiscalus quiscula*) has also been studied (Walley, 1965), and 90% of the capsule-fed DDT was recovered in a 3-day feces sample. In addition to DDT, DDE and DDD were found in liver, brain, and breast muscle. Cross *et al.* (1962) have found that levels of up to 300 ppm of DDT in the diet did not affect the weight or number of eggs laid by Japanese quail (*Coturnix coturnix japonica*). With diet containing 500 ppm the eggs failed to hatch and the birds died before the tests were completed. Domestic poultry fed DDT at levels up to 250 ppm in the diet for 15 weeks, showed no clinical toxic effects or loss of condition (Noakes and Benfield, 1965). DDT, DDE, and DDD were found in the tissues in amounts roughly proportional to the dosage levels. There was a general trend toward decrease of DDD and increase of DDE in all tissues. Only a limited amount of work has been carried out *in vitro* in avian tissue, where Bunyan *et al.* (1966) demonstrated that DDD is the major metabolite under anaerobic conditions whereas DDE is produced to a lesser extent under aerobic conditions in the presence of glutathione. We now wish to report our findings on the feeding of DDT to feral pigeons (*Columba livia*). Although the feeding level employed gives rise to higher residues than those normally found in wild birds in England, this level was chosen to produce residues over long periods and in amounts adequate for identification.

EXPERIMENTAL

DDT was prepared from technical DDT by the method of West and Campbell (1950) and finally recrystallized from ethanol to constant melting point 108–109° (uncorr.). Only one peak was obtained by gas-liquid chromatography. English wheat was dressed with this DDT at a level of 1000 ppm using 0.5% technical white oil as a sticker. All diets were subsequently sampled and analyzed by gas-liquid chromatography to check that levels of dressings had been achieved. Thirty-six weighed pigeons were separately

caged and fed this dressed grain for 24 days. Six control birds were fed the same undressed wheat for the same period. Pigeons were stratified according to weight and randomly selected from a large group of feral birds of unknown history and, owing to the virtual impossibility of sexing this species, the proportion of males and females was not known until the animals were dissected. The DDT-fed birds comprised 18 males and 18 females, and the control group contained 3 male and 3 female birds. Before and after feeding wheat, the birds were communally housed and fed a diet of mixed grain and pulses. The pigeons were sacrificed in batches of six at 1, 29, 57, 85, 113, and 274 days after the withdrawal of the dressed grain. Control birds were killed at intervals during this period. Analyses were carried out on breast muscle, liver, kidney, brain, heart, gonads (ovaries or testes), and omental fat. The method of Taylor *et al.* (1964) was used for the extraction of tissues prior to gas-liquid chromatographic analysis with all-glass injection and column system. Other conditions were as described by Goodwin *et al.* (1961). DDT, DDD [DDE plus 1,1-di(*p*-chlorophenyl)-2-chloroethane (DDMS)], 1,1-di(*p*-chlorophenyl)-2-chloroethylene (DDMU), and 1,1-di(*p*-chlorophenyl)ethylene (DDNU) could be separated on silicone epikote columns. DDE and DDMS which have the same retention value separated from each other on a 2.5% QF1/0.25% epikote column. The lower limit of detection of these compounds was of the order of 0.01 ppm. Thin-layer chromatography was used for confirmation of identity. One bird died during the course of the experiment, but owing to unforeseen circumstances it was not analyzed.

A similar experiment involving 18 birds (8 males and 10 females) with another control group (1 male and 5 females) was performed feeding wheat dressed with DDD at 1000 ppm for 24 days. The DDD was prepared from technical Rhothane by recrystallization from methanol to constant melting point 109–110° (uncorr.). Gas-liquid chromatographic analysis gave only one peak. Birds were sacrificed in batches of 6 at 1, 29, and 92 days after the cessation of feeding treated grain. The control group was sacrificed after 120 days. No deaths occurred in any of the groups and the birds were sampled and dealt with as described for the DDT-fed birds.

The number of analyses involved in these experiments necessitated tissue storage. Tissues were removed from all birds immediately following sacrifice and subsequently kept below 0°C until analyses were carried out between 1 and 14 days later. Experiments to be reported elsewhere using pigeons injected with DDT and its metabolites have demonstrated that all residues reported in this communication are present immediately after death and are therefore not due to postmortem bacterial action. Furthermore although some changes were observed on storage of dissected tissue below 0°, they were both small and erratic, and probably lie within the experimental error of the method. No underlying trend of the type reported by Jefferies and Walker (1966) for Bengalese finches (*Lonchura striata*) could be found in pigeons.

RESULTS

Clearly identifiable continuous tremoring was observed in a high proportion of birds after 10 days' feeding of DDT, but no effects were noted with DDD. Birds on the diet of DDT consumed an average of 456 ± 13 g (M ± SEM) of wheat per bird over the 24-day period compared with the control group's consumption of 603 ± 27 g. No

individual consumption differed significantly from these values. In the case of DDD the difference in consumption was minimal at 555 ± 22 g for the treated and 528 ± 42 g for the control group. In neither experiment was the food consumption in the treated group significantly different from that of the controls.

The increase in liver weight which has been noted in rats fed organochlorine pesticides (Chen-Yu and Yuan Peng, 1966; Fitzhugh and Nelson, 1947; Laug and Fitzhugh, 1946; Sarett and Jandorf, 1946, 1947) was confirmed in the pigeons fed DDT. The maximum increase in liver size was not reached until the birds had been on normal food for 1–2 months. The average pigeon liver weight from a large number of control birds was 8 ± 0.4 g. With treated birds the maximum average which occurred after 1 month was 29 ± 3.7 g and at the end of our experiment was still 12 ± 1.0 g. Both these weight increases are highly significant ($p < 0.001$). With the DDD-fed birds the increase in liver weight was much less. Only in the batch following the cessation of feeding was a significant ($p < 0.05$) increase to 10.6 ± 1.05 g found. By the end of the experiment the liver weight was back to normal.

In the birds given DDT-treated food, DDT, DDE, and DDD were found at some time in all the organs examined with the exception of DDD in adipose tissue. Despite the wide range of results encountered in each batch of birds it was evident that the amounts of each of these compounds decreased in the tissues examined as the time between cessation of diet and the sacrifice of the birds increased, and that the relationship between the amounts and the time was not linear. Satisfactory linear relationships were first obtained graphically by transforming the ppm of compounds to $\log_e$ (100 ppm + 1). This transformation allowed the necessary inclusion of nil readings without introducing error, by making an addition small in magnitude with respect to all other residue levels measured. The linear regressions of the transformed concentrations of each compound on the times from cessation of diet, calculated separately for each organ and tissue, then fitted significantly well. The regression coefficients (b), are given in Table 1, which also shows the results of comparisons between the seven coefficients for each compound together with appropriate half-lives and their 95% fiducial limits. The constants (a) are also given for each tissue since they are estimates of $\log_e$ (100 C_0 + 1) where C_0 is the concentration of DDT or its metabolites when exposure ceased. By the use of natural logarithms in our transformation and substitution into the linear regression equation

$$Y = bx + a$$

we arrive at the equation

$$(C_t + 0.01) = 0.01)e^{-bt}$$

Thus b becomes also the best available estimate from our normally variable data of the rate constant (k) in the more familiar equation

$$C_t = C_0 e^{-kt}$$

In the case of DDT and DDE residues no significant difference was exhibited between the regression coefficients for the seven tissues. Consequently a common regression coefficient of −0.024 describes the rate of elimination or change of DDT, and a common regression coefficient of −0.005 describes the rate of elimination or change of DDE in all the sites considered. Partitioning the significant variance for the regression

The authors regret the following errata:-

Page 16. line 36. equation should read:-

$$(C_t + 0.01) = (C_0 + 0.01)e^{-bt}$$

Page 17. Subscript a should read:-

[a]Each bird consumed an average of 456 ± 13 mg of DDT during the 24 days feeding.

Page 18. Subscript a should read:-

[a]Each bird consumed an average of 555 ± 22 mg of DDD during the 24 days of feeding.

Page 21. line 15. delete comma insert stop at end of sentence.

Page 23. Abstract, lines 5 & 6 should read:-

the halogenated hydrocarbons, 1,1-di(p-chlorophenyl)-2,2-dichloroethylene (DDE) and 1,1-di(p-chlorophenyl)-2-chloroethylene (DDMU)

Page 29. line 9 organs not organic

Throughout both papers in the text (Bailey et al., 1968) should read (Bailey et al., 1969)

TABLE 1

TRANSFORMED CONCENTRATIONS OF DDT, DDD, AND DDE IN SEVEN BODY TISSUES OF PIGEONS AS A FUNCTION OF TIME AFTER TERMINATION OF EXPOSURE TO DDT[a]

Tissue	Regression line $y = bt + a$: Regression coefficient, b	Regression line $y = bt + a$: Constant a[b]	Compound	Analysis of covariance[c]
1. Breast muscle	−0.025	5.62	DDT	Source of variation: df: MS: F
2. Liver	−0.020	4.24		Between coefficients: 6: 10.88: 1.22 NS
3. Kidney	−0.022	5.28		Within regressions: 238: 8.90: —
4. Brain	−0.018	3.86		Common regression coefficient for all tissues: −0.024***
5. Heart	−0.027	5.82		Half-life (M) of DDT and 95% fiducial limits:
6. Gonads	−0.022	4.96		M: L.L.: U.L.
7 Omental fat	−0.037	8.23		28 days: 7 days: 45 days
				Source of variation: df: MS: F
				Between coefficients: 5: 14.18: 3.48**
				Between b_1, b_2, b_3, b_5: 3: 10.00: 2.45 NS
				Between b_4, b_6: 1: 1.21: <1.0 NS
1. Breast muscle	−0.015	2.93		$b_1 + b_2 + b_3 + b_5$ vs. $b_4 + b_6$: 1: 39.67: 9.75**
2. Liver	−0.025	4.90		Within regressions: 204: 4.07: —
3. Kidney	−0.018	3.40		Common regression coefficient for tissues 1, 2, 3, 5: −0.017***
4. Brain	−0.005	0.98	DDD	Half-life (M) of DDD and 95% fiducial limits:
5. Heart	−0.010	1.91		M: L.L.: U.L.
6. Gonads	−0.008	1.50		41 days: 10 days: 64 days
7. Omental fat	—	0.00		Common regression coefficient for tissues 4, 6: −0.007***
				Half-life (M) of DDD and 95% fiducial limits:
				M: L.L.: U.L.
				102 days: 56 days: 155 days
1. Breast muscle	−0.004	8.80	DDE	Source of variation: df: MS: F
2. Liver	−0.009	9.44		Between coefficients: 6: 0.73: <1.0 NS
3. Kidney	−0.004	8.19		Within regressions: 238: 0.85: —
4. Brain	−0.006	7.08		Common regression coefficient for all tissues: −0.005***
5. Heart	−0.005	9.13		Half-life (M) of DDE and 95% fiducial limits:
6. Gonads	−0.006	7.86		M: L.L.: U.L.
7. Omental fat	−0.004	12.06		128.6 days: 108 days: 154 days

[a] Each bird consumed an average of 555 ± 22 mg of DDT during the 24 days of feeding.
[b] $a = \log_e (100\ C_0 + 1)$. C_0 is the best available estimate of the concentration of DDT or metabolite when exposure to DDT ceased.
[c] NS, Not significant; ** significant at $p < 0.01$; *** significant at $p < 0.001$; L.L., lower limit; U.L., upper limit.

TABLE 2

TRANSFORMED CONCENTRATIONS OF DDD, DDMU, AND DDE IN SEVEN BODY TISSUES OF PIGLONS AS A FUNCTION OF TIME AFTER TERMINATION OF EXPOSURE TO DDD[a]

Tissue	Regression line $y = bt + a$: Regression coefficient b	Regression line $y = bt + a$: Constant, a[b]	Compound[c]	Analysis of covariance[d]
1. Breast muscle	−0.026	8.29	DDD	Source of variation: df / MS / F Between coefficients: 6 / 0.66 / <1.0 NS Within regressions: 112 / 0.94 / — Common regression coefficient for all tissues: −0.029*** Half-life (M) of DDD and 95% fiducial limits: M: 23.8 days; L.L.: 17 days; U.L.: 30 days
2. Liver	−0.032	7.80		
3. Kidney	−0.028	7.22		
4. Brain	−0.034	5.93		
5. Heart	−0.031	8.49		
6. Gonads	−0.020	6.59		
7. Omental fat	−0.032	11.25		
1. Breast muscle	−0.003	6.32	DDMU	Source of variation: df / MS / F Between coefficients: 6 / 0.98 / 1.10 NS Within regressions: 112 / 0.89 / — Common regression coefficient for all tissues: −0.008*** Half-life (M) of DDMU and 95% fiducial limits: M: 90 days; L.L.: 77 days; U.L.: 173 days
2. Liver	−0.007	5.84		
3. Kidney	−0.011	5.30		
4. Brain	−0.017	4.26		
5. Heart	−0.013	6.80		
6. Gonads	−0.002	4.86		
7. Omental fat	−0.003	8.38		

[a] Each bird consumed an average of 456 ± 13 mg of DDD during the 24 days of feeding.
[b] $a = \log_e (100\ C_0 + 1)$. C_0 is the best available estimate of the concentration of DDD or metabolite when exposure to DDD ceased.
[c] Small amounts of DDE were found throughout the experiment (range 0-2 ppm in all tissues except fat 2-64 ppm), but no significant regression coefficients were obtained. Comparable control figures were 0-1 ppm in all tissues.
[d] N.S., Not significant; ** significant at $p < 0.01$; *** significant at $p < 0.001$; L.L., lower limit; U.L., upper limit.

coefficients for DDD into orthogonal components reveals two different rates of loss. That in liver, kidney, muscle, and heart ($b = -0.017$) is significantly faster ($p < 0.01$) than that in brain and gonads ($b = -0.007$).

In the birds given DDD-treated food, DDD and DDMU were found in all the tissues examined including fat, in relatively large amounts for the duration of the experiment. Small residues of DDE, which were generally higher than those in control birds, were found in all tissues, and in a few birds residues of DDMS were also noted. In the first group DDMS residues (0.3-6.2 ppm) were confined to the kidney. In the last two groups a residue of 3.2 ppm was found in the brain of one bird and a residue of 3.0 ppm in the liver of another bird. The statistical treatment previously described for the data from DDT-fed birds was again carried out to give the regression coefficients, constants, half-lives, and analyses in Table 2.

A comparison of the regression coefficients shows that there are no significant differences in the rates of elimination or change of either DDD or DDMU in the seven organs. Regression coefficients of −0.029 for DDD and −0.008 for DDMU are common to all seven organs. In the case of DDE residues the calculated regressions did not fit the data significantly.

DDE (<1 ppm) was the only residue found in control birds.

DISCUSSION

A consideration of these results indicates that neither DDT nor DDD is particularly toxic to the pigeon, nor do they seriously affect the palatability of the food treated at a level of 1000 ppm. The induction of liver weight increases (*loc. cit.*), and stimulation of hepatic microsomal enzymes (Gerboth and Schwabe, 1964; Kinoshita *et al.*, 1966) by DDT has been reported mainly in rats. In general it has been tacitly assumed that the causative agent is DDT, and not a metabolic product, although it has recently been pointed out (Hart and Fouts, 1965) that in the rat the metabolites are as effective. These authors have also drawn attention to the species differences in the response of the liver to DDT and its metabolites. Our results reveal that the feeding of DDT to the pigeon produces considerable amounts of DDE which persist after other residues have disappeared. This suggests that DDE may be the true causative agent for liver weight increase in the pigeon. The fact that this increase does not reach a maximum until some time after the cessation of feeding and that liver weights have not returned to normal 11 months later, when the liver contains no other residue than DDE, accord well with this theory. It is also significant that in DDD-fed birds where DDE residues are very small and there is no other persistent large residue, liver weights are scarcely affected. These conclusions are firmly supported by results from birds fed DDE which are the subject of a separate communication (Bailey *et al.*, 1968).

Residue measurements on the DDT-fed pigeons show that DDT is lost fairly rapidly from all organs (Table 1). DDE and DDD appear to be the two major metabolic products in the pigeon as in the rat (Peterson and Robison, 1964). In view of the fact that DDE residues remain in the latter groups of birds where no other residues are found, it seems likely that DDD and DDE are produced by separate pathways. This is further supported by results obtained from feeding both DDD, when very small residues of DDE were found, and DDE (Bailey *et al.*, 1968) where no DDD residues were detected.

Furthermore, the longevity of DDE residues suggests that it is an extremely difficult compound for the bird to eliminate.

The DDD residues produced in the DDT-fed birds present the most interesting aspect of the experiment. The production of DDD from DDT by vertebrates has been questioned. It has been demonstrated (Barker and Morrison, 1964) in mice that DDD is formed only as a result of postmortem breakdown of DDT. Jefferies and Walker (1966) report a similar phenomenon in Bengalese finches, and it has also been shown (Barker *et al.*, 1965) that *Proteus vulgaris* from the gut of mice could convert DDT to DDD. However, earlier work in this laboratory (Bunyan *et al.*, 1966) on pigeon liver systems *in vitro* suggests that DDD is a primary metabolic product under anaerobic conditions. DDD was also demonstrated as a metabolic product after feeding of DDT to rats (Datta *et al.*, 1964) and this conversion has been reported (Morello, 1965) using rat liver microsomes with molecular oxygen and NADPH as requirements. Results from our feeding experiments support the view that DDD is a true metabolic breakdown product of DDT in pigeon tissue and there appear to be two significant rates of loss of DDD among the seven tissues examined (Table 1). This metabolite was not found in fat at all. Furthermore brain and gonads whose rate of loss of DDD is significantly slower than the other tissues also have high lipid content. With all other residues investigated there is a common regression coefficient for all tissues, although the constants (*a*) differ. These results indicate that a dynamic equilibrium exists between the tissues due to circulation in the blood from a primary metabolic site which is generally assumed to be the liver. This parallels the findings of Robinson *et al.* (1967) for dieldrin residues in pigeons. In the case of DDD produced from DDT however, tissues rich in lipid differ from the normal model. No reasonable explanation of this phenomenon can currently be offered, although more should be known about possible alternative metabolic sites for these compounds. Metabolism has so far been demonstrated with certainty only in liver, but it may be that adipose tissue plays a more dynamic role in DDT metabolism than hitherto suspected. Rumsey *et al.* (1967) have also noted some peculiarities in distribution of residues in various types of beef fat following DDT feeding.

While we do not exclude the possibility of DDD being produced by gut flora, had this been the only source, residues would have been found in fat and a common regression coefficient for all organs would have been obtained as was the case when DDD was fed. Furthermore large-scale postmortem production of DDD has been eliminated, as detailed previously.

The birds fed DDD-treated food show residues in all tissues and appear to be able to metabolize the pesticide rapidly and exclusively to DDMU. The metabolic pathway suggested for the rat (Peterson and Robison, 1964) would therefore seem to be common to the pigeon at least as far as DDMU. The common regression rate of −0.029 for DDD is very similar to that for DDT in birds fed this compound, and suggests that both are dealt with readily. The lower rates of DDD loss obtained from DDT feeding are probably due to the continuous production of DDD from DDT. Despite the common regression rate (−0.008) of DDMU, very little DDMS was found—and this mainly in the kidney at an early stage of the experiment. This suggests that the metabolic pathway of DDT in the pigeon may diverge from that in the rat after the production of DDMU. The small quantities of DDE found after DDD feed-

ing are slightly larger than control residues, but they do not indicate a major metabolic pathway.

From an overall consideration of these results it would appear that the pigeon is able to metabolize DDT and DDD quite rapidly and is not affected even by the massive quantities that we have fed. The production of DDE and DDMU, however, may complicate the situation since these seem to be lost more slowly and no further metabolic products from these compounds have been demonstrated in this work. It has been shown (Smith *et al.*, 1946) that DDE is less toxic to rats than DDT, and it has been suggested recently that the avian toxicity of DDE is low (Walker *et al.*, 1967). However, in view of the species differences known to exist in the response to DDT and its metabolites (Hart and Fouts, 1965), the storage of DDE may be more harmful than previously suspected.

ACKNOWLEDGMENTS

Gifts of technical DDT from the Murphy Chemical Co. Ltd. and technical DDD from the Lennig Chemical Co. Ltd. are gratefully acknowledged. We are indebted to Dr. E. E. Turtle for helpful discussion and to Mr. F. Jones, Mr. D. Summers, and Miss G. Parrott for the considerable work involved in feeding, observing, housing, and dissecting the birds. The several hundred analyses were ably performed by our colleagues Mrs. M. Nicholls, Miss L. Lockhart, and Miss C. Hewitt.

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